Serologic Evidence for Exposure to Simian Virus 40 in North American Zoo Workers

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(See the editorial commentary by Shah, on pages 2061–4.)

Some laboratories have detected DNA from the macaque polyomavirus simian virus 40 (SV40) in human tumors, but possible routes of infection remain unknown. In the present study, an enzyme immunoassay using viruslike particles (VLPs) was used to test 254 zoo workers for antibodies to SV40; 25 zoo workers with direct contact with nonhuman primates and 15 other zoo workers (23% vs. 10%, respectively; $P = .01$) were seropositive for SV40. Additionally, SV40 seroreactivity confirmed by competitive-inhibition experiments (i.e., blocked by addition of SV40 VLPs but not by VLPs for BK virus or JC virus, which are related human polyomaviruses) was increased in zoo workers with direct contact with nonhuman primates (10% vs. 3%, respectively; $P = .04$). SV40 seroreactivity therefore may reflect zoonotic exposure.

The macaque polyomavirus simian virus 40 (SV40) was a contaminant of inactivated poliovirus vaccine produced in monkey kidney tissue and used widely in the United States during 1955–1962 [1]. This exposure to SV40 is important because SV40 causes cancer in laboratory rodents. Some investigators have reported detection of SV40 DNA in various human tumors [2]. One difficulty in the interpretation of reports of detection of SV40 sequences in patients with cancer is that both the prevalence of SV40 in humans and its possible transmission routes remain unknown [2]. On the basis of limited data, the seroprevalence of SV40 in the general US and European populations has been inferred to be ~5%–10% [3–6]. Two SV40-related human polyomaviruses, BK virus (BKV) and JC virus (JCV), are acquired early in life, are highly prevalent, and establish lifelong infection, frequently accompanied by a robust antibody response [4, 7]. SV40 establishes a similarly persistent infection in macaques [1], but SV40-seropositive humans exhibit only low-level antibodies to SV40, which might represent cross-reactive responses to either BKV or JCV [4, 6, 7].

Recently developed EIAs that detect antibodies to SV40 by using viruslike particles (VLPs) provide a valuable tool for investigation of the epidemiology of SV40 [4, 6, 7]. VLPs are empty capsids formed by spontaneous self-assembly of the VP1 major-capsid protein. SV40 VLPs resemble native virions morphologically and antigenically, making them ideal reagents with which to detect antibodies to surface-exposed viral epitopes. Furthermore, competitive-inhibition experiments using this assay can address whether SV40 seroreactivity is specific or might instead be due to cross-reactive responses to either BKV or JCV [4, 6, 7].

Individuals who work with nonhuman primates may be at risk for occupationally acquired SV40. SV40 is highly prevalent among rhesus macaques, and other species of Old World monkeys are frequently infected in captivity [1]. SV40 is shed in macaque urine [8]. Zoo workers, veterinarians, and laboratory workers might acquire animal viruses through bites, scratches, or mucocutaneous exposures. SV40 can infect human cells in vitro, suggesting that humans are at risk for cross-species infection with SV40 [2]. Zoonotic transmission from nonhuman primates to humans is well-documented for other viruses—for example, herpesvirus B, simian immunodeficiency virus, and simian foamy virus [9–12].

In the present study, we examined the seroprevalence of SV40 in zoo workers with occupational exposure to nonhuman primates. We performed serological testing for SV40 by using a VLP-based EIA, and we evaluated the specificity of any observed SV40 seroreactivity in competitive-inhibition experiments. We report here an elevated prevalence of SV40 seroreactivity in individuals who work with nonhuman primates.

Subjects and methods. We evaluated 254 North American zoo workers who participated in an anonymous serosurvey during 1997 [9]. In accordance with human-experimentation guidelines of the US Department of Health and Human Services, these workers provided informed consent for participa-

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tion in the study. As part of the study, the workers provided a brief job description and, before analysis of serologic data, were classified with respect to exposure to nonhuman primates [9]: "nonhuman-primate zoo workers" ($n = 109$) comprised both those currently working specifically with either nonhuman primates or a larger class of animals including nonhuman primates and those in senior administrative positions, which were assumed to be filled by individuals with extensive animal-handling experience; "other zoo workers" ($n = 145$) comprised both those currently working with classes of animals not including nonhuman primates and those performing maintenance, clerical, or visitor service. In some analyses, we subdivided nonhuman-primate zoo workers into those with frequent/ongoing exposure (i.e., nonhuman-primate keeper, nonhuman-primate laboratory technician, or veterinarian/veterinarian assistant; $n = 71$) and those with only infrequent/past exposure (i.e., the remaining nonhuman-primate zoo workers; $n = 38$). No other demographic or occupational data were available.

For the EIA experiments, SV40 VLPs, BKV VLPs, and JCV VLPs were generated from recombinant baculoviruses expressing VP1 major capsid protein [7]. EIA plates (PolySorp; Nunc) were coated, overnight at 4°C, with 20–30 ng/well of VLP in PBS (pH 7.2) and were blocked, for 3 h at room temperature, with 0.5% (wt/vol) polyvinyl alcohol (PVA) in PBS. Before use and after each incubation step, EIA plates were washed 4 times with PBS containing 0.05% (vol/vol) Tween 20 (Sigma).

The testing laboratory received serum samples that were coded (i.e., masked with respect to their donors' occupations). Specimens were diluted 1:400 in 0.5% PVA and were applied to EIA plates. After incubation for 1 h at 37°C, antigen-bound immunoglobulin was detected by peroxidase-conjugated goat antibodies against human IgG (Zymed) diluted 1:4000 in 0.5% PVA, 0.0025% Tween 20, and 0.8% (wt/vol) polyvinylpyrrolidone (Sigma). After incubation for 30 min at 37°C, 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution (Kirkegaard and Perry) was added to plates. After 20 min, optical density (OD) at 405 nm was measured by use of an automated plate reader. The geometric mean of measurements in duplicate specimens was used in the analyses. For the SV40 EIA, a cutoff of 0.10 OD units was chosen, on the basis of both inspection of a histogram of the results and consistency with prior results in serum samples from macaques and humans; the same cutoff was used for the BKV and JCV EIAs. Because there is no reference standard for SV40 infection in humans, the SV40 EIA's sensitivity and specificity in humans is unknown; in macaques, however, this assay demonstrates ~100% sensitivity and ~100% specificity for SV40 infection [6].

To evaluate the specificity of EIA-measured SV40 seroreactivity in the zoo workers in the present study, SV40-seropositive serum samples were tested in competitive-inhibition experiments. Specifically, serum samples were diluted 1:200, 1:400, or 1:800 (depending on the initial OD in the SV40 EIA), either in 0.5% PVA containing a 4-μg/mL concentration of either SV40 VLPs, BKV VLPs, or JCV VLPs or in 0.5% PVA alone. Serum samples were incubated, for 1 h at 37°C, on SV40-VLP EIA plates, and the EIA was then completed as described above. SV40 seroreactivity was considered to be "competitively inhibited" (i.e., blocked or absorbed) by a VLP if the SV40 EIA–based OD for serum preincubated with that VLP was <50% of that for serum without competing VLP. "SV40-specific" seroreactivity was then defined as SV40 seroreactivity that was competitively inhibited by SV40 VLPs but not by BKV VLPs or JCV VLPs. With this approach, EIA-measured SV40-VLP seroreactivity in macaques can be shown to be SV40 specific, and similar competitive-inhibition experiments have demonstrated the specificity of EIA-measured BKV-VLP seroreactivity and JCV-VLP seroreactivity in humans ([4, 7] and authors' unpublished data).

Results. Most zoo workers exhibited only low-level SV40 seroreactivity (figure 1). When an EIA cutoff of 0.10 OD units was used, 23% of nonhuman-primate zoo workers and 10% of other zoo workers were SV40 seropositive ($P = .01$, table 1). In comparison, most zoo workers were BKV and JCV seropositive (figure 1), and seroprevalence did not differ between nonhuman-primate zoo workers and other zoo workers (table 1). Results were similar when the EIA cutoff was varied slightly (data not shown).

SV40 seroreactivity showed modest positive correlation with BKV seroreactivity (Spearman's $R = 0.32$; $P < .0001$) and with JCV seroreactivity (Spearman's $R = 0.23$; $P = .0002$). Among SV40-seropositive zoo workers, SV40 seroreactivity was low (median SV40 EIA–based OD, 0.17), whereas both BKV seroreactivity and JCV seroreactivity were much higher (median BKV EIA–based OD in BKV-seropositive zoo workers, 0.65; median JCV EIA–based OD in JCV-seropositive zoo workers, 0.45) (figure 1).

The 40 SV40-seropositive zoo workers were tested in competitive-inhibition experiments; of the 29 zoo workers for whom evaluable results were available, 14 were considered to have SV40-specific seroreactivity, and 15 were considered to have SV40-nonspecific seroreactivity (table 1). Among evaluable subjects, the proportion of zoo workers demonstrating SV40-specific seroreactivity was higher in nonhuman-primate zoo workers than in other zoo workers (10% vs. 3%, respectively; $P = .04$).

In the 2 groups of nonhuman-primate zoo workers—that is, those with frequent/ongoing exposure to nonhuman primates and those with infrequent/past exposure to them—the EIA-measured SV40 seroprevalence was similar (20% vs. 29%, respectively; $P = .39$), as was the prevalence of SV40-specific seroreactivity (9% vs. 12%, respectively; $P = .73$). SV40-spe-

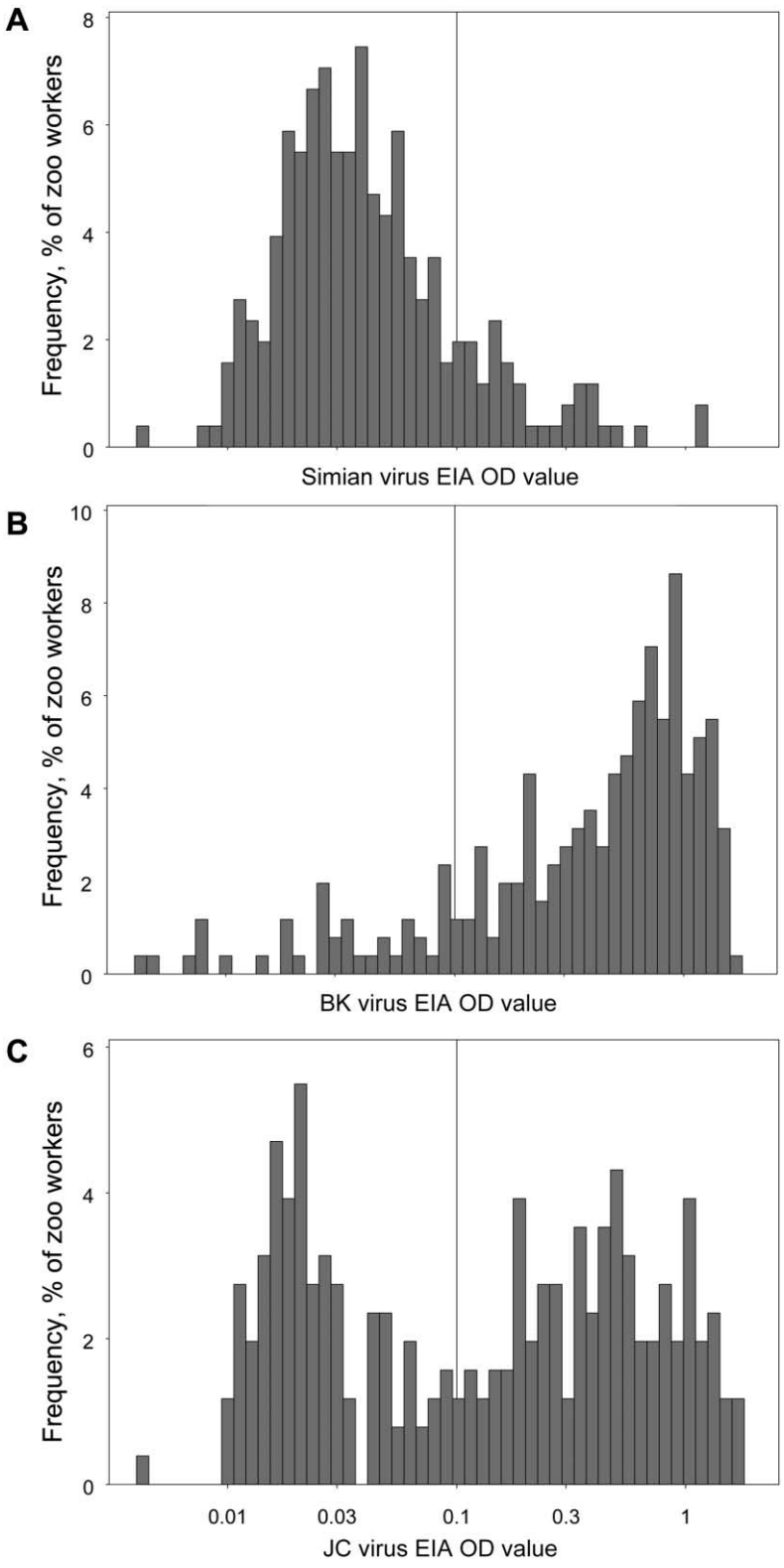

Figure 1. Antibodies to 3 polyomaviruses—simian virus 40 *(A),* BK virus *(B),* and JC virus *(C)*—in 254 North American zoo workers. The geometric mean of 2 optical density (OD) measurements derived by use of a viruslike-particle–based EIA are depicted as histograms. OD measurements are grouped on a logarithmic scale; the vertical line indicates the cutoff (0.10 OD units) used to define a seropositive result. Note that the vertical scales differ in the 3 panels.

Table 1. Antibody to simian virus 40 (SV40) and to BK virus (BKV) and JC virus (JCV): serostatus in zoo workers exposed to nonhuman primates and in other zoo workers.

Polyomavirus serostatus	Nonhuman-primate zoo workers (N = 109),[a] no. (%)	Other zoo workers (N = 145)[b], no. (%)
SV40 seropositive	25 (23)	15 (10)
Competitively inhibited		
By SV40 but not by either BKV or JCV (i.e., SV40 specific)	10 (9)	4 (3)
By SV40 and by BKV and/or JCV	1 (1)	4 (3)
Not competitively inhibited by SV40	6 (6)	4 (3)
No data on competitive inhibition	8 (7)	3 (2)
SV40 seronegative	84 (77)	130 (90)
BKV seropositive	93 (85)	123 (85)
JCV seropositive	61 (56)	81 (56)

NOTE. VLP, viruslike particle.

[a] Individuals currently working specifically with either nonhuman primates or a larger class of animals including nonhuman primates, as well as individuals in senior administrative positions, who were assumed to be individuals with extensive animal-handling experience.

[b] Individuals currently working with classes of animals not including nonhuman primates, as well as individuals performing maintenance, clerical, or visitor service.

cific seroreactivity was observed in 6 nonhuman-primate zoo workers with frequent/ongoing exposure to nonhuman primates (5 nonhuman-primate keepers and 1 nonhuman-primate laboratory technician), in 4 nonhuman-primate zoo workers with infrequent/past exposure (1 zoo supervisor, 1 senior animal keeper, 1 mammal keeper, and 1 animal researcher), and in 4 other zoo workers (1 curator, 1 electrician, 1 maintenance worker, and 1 park-attendance worker). The only zoo worker with a job description specifically involving care of rhesus monkeys ("rhesus colony manager") was SV40 seronegative.

Discussion. Among zoo workers in the present study, EIA-measured SV40-VLP seroprevalence was significantly elevated in those who worked with nonhuman primates. This increase remained after an added level of stringency—that is, use of competitive-inhibition data to eliminate possible false-positive SV40 seroreactivity from either BKV or JCV—was incorporated. The results of the present study complement those which Shah provided in a 1966 study of workers at 2 monkey-export firms in northern India [13]; in that investigation, 10 (27%) of 37 workers exhibited SV40-neutralizing antibodies, generally at low titers (median titer, 1:8). The prevalence of SV40-neutralizing antibody increased with duration of service, from 6% in those with <6 years of employment, to 31% in those with 6–10 years of employment, and to 71% in those with 11–13 years of employment ($P_{\text{trend}} = .005$, calculated by the authors of the present study). Together, these 2 studies provide evidence that humans working closely with nonhuman primates are occupationally exposed to SV40.

A limitation of our serologic data (and of those reported by Shah [13]) is that they could not definitively distinguish among the following 3 biologically possible explanations for the presence of SV40 antibody in humans: (1) prior immunizing exposure to SV40, without persisting infection; (2) persisting SV40 infection of cells in 1 or more types of tissue, without completion of the viral life cycle (i.e., "nonpermissive infection") [14]; and (3) persistent SV40 infection with viral replication. In their natural hosts, polyomaviruses (such as BKV and JCV in humans and SV40 in macaques) manifest the last of these possibilities: the viruses establish lifelong latent infections in the kidney, and viral DNA can be detected in urine and peripheral-blood mononuclear cells (PBMCs), even from immunocompetent individuals [15]. Probably as a consequence of ongoing or intermittent low-level viral replication, most BKV- or JCV-infected persons and most SV40-infected macaques mount readily detectable antibody responses against viral capsid proteins [4, 7]. However, despite the ability of SV40 to infect human cells in vitro [2], it remains unclear whether humans can actually be infected with SV40. Published data on the molecular detection of SV40 in peripheral blood, urine, and tumor tissue are conflicting [2], and the present study lacked the PBMCs and urine samples that would be necessary to examine this question directly. The mostly low-level SV40 seroreactivity observed in both the present study and earlier studies [4–7, 13] can be interpreted as suggesting that there is no ongoing replication of SV40. Because there were no available data on the zoo workers' health status, either when serum samples were obtained or during follow-up, the present study could not evaluate associations between SV40 serostatus and disease (e.g., cancer).

The results of the present study do not provide a reliable estimate, in absolute terms, of the risk, to nonhuman-primate zoo workers, of exposure to and/or infection with SV40. If the level of SV40 antibody declines over time because of an absence of either repeated exposure to SV40 or ongoing replication of

SV40, then the present study's estimates of seroprevalence might underestimate the proportion of nonhuman-primate zoo workers ever exposed to SV40. Furthermore, the present study provided little or no information with regard to either the specific nonhuman primates to which zoo workers were exposed or the duration of time that they worked with these nonhuman primates. Finally, there were no data on occupational injuries or specific incidents—such as bites, scratches, or splashes—that may have led to transmission of SV40. Considered together, these difficulties may explain why there was no difference between the SV40 seroprevalence in zoo workers classified as having frequent/ongoing exposure and that in those with infrequent/past exposure to nonhuman primates—and why the rhesus-colony manager was SV40 seronegative.

Both the results of the present study of zoo workers unexposed to nonhuman primates and the results of other studies of persons unexposed to nonhuman primates [3–6] suggest that the prevalence of SV40 infection in the general population is low—only 10% of zoo workers unexposed to nonhuman primates were SV40-VLP seropositive. However, although EIA-measured seropositivity is ~100% specific for SV40 infection in macaques [6], the assay's specificity in humans may be lower. Indeed, as evidenced both by the correlations between the EIA results for SV40 and those for BKV and JCV and by the results of the competitive-inhibition experiments, much of the SV40 seroreactivity in the zoo workers in the present study was probably due to BKV or JCV cross-reactivity. Alternatively, nonspecific SV40 seroreactivity could have been caused by infection with another nonhuman-primate polyomavirus [14]. However, the present study did not test for the latter, and the lack of association between nonspecific SV40 seroreactivity and exposure to nonhuman primates would argue against this possibility (table 1). In some individuals, SV40 seroreactivity could reflect prior exposure to SV40-contaminated poliovirus vaccines [1]; the present study did not have data on the zoo workers' ages, but it is likely that many were born before 1963.

In conclusion, the results of the present study suggest that those who work closely with nonhuman primates are occupationally exposed to SV40. Further studies with larger numbers of persons exposed to nonhuman primates will be necessary to better estimate the risk of exposure, identify routes of SV40 transmission, characterize whether infection is abortive or persistent, and determine whether there are health consequences of SV40 infection.

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